REMARKS/ARGUMENTS

In response to the Office Action of January 3, 2006, Applicants request re-examination and reconsideration of this application for patent pursuant to 35 U.S.C. 132.

Claim Status/Support for Amendments

Claims 1, 36, 37, and 41-43 have been amended. Claims 2-35 were cancelled in a previous Response filed on June 13, 2003. Claims 1 and 36-43 are under examination and remain pending in the instant application.

Claim 1 has been amended to clarify that the claimed marker is "isolated"; see the instant specification as originally filed at page 31, lines 9-12 . Additionally, the use of the claimed marker, diagnostic for myocardial infarction (MI), intracerebral hemorrhage (ICH) or congestive heart failure (CHF), has been removed from claim 1.

Claim 36 has been amended to indicate how the presence of the claimed marker is determined from mass spectral profiles; see the instant specification as originally filed at page 26, line 20 to page 28, line 2 and Figures 1 and 2.

Claim 37 has been amended to provide proper antecedent basis for the term "sample".

Claim 41 has been amended to indicate that samples are

obtained from patients; see page 26, line 20 to page 27, line 2 and Figure 1 of the instant specification as originally filed.

Claims 42 and 43 have been amended to provide proper antecedent basis for the term "diagnostic kit".

Rejection under 35 USC 101

Claim 1, as presented on May 16, 2005, stands rejected under 35 USC 101 because the claimed invention is allegedly directed to non-statutory subject matter.

The Examiner asserts that the amino acid residues 2-12 of SEQ ID NO:1 are specified, for example to be "isolated" and/or "purified" and therefore do not indicate the hand-of-man.

Claim 1 has been amended herein to recite the biopolymer marker as "isolated" which distinguishes the marker from those polypeptide sequences found in nature. The term "isolated" is interpreted to mean "altered by the hand of man" from its natural state; for example, if it occurs in nature and is then "isolated", it has been changed or removed from its original environment or both. A biopolymer, such as that claimed herein (amino acid residues 2-12 of SEQ ID NO:1), naturally present in a living organism is not "isolated", however, the same biopolymer separated from the co-existing materials of its natural state is "isolated". It is clear from the methods recited herein that the claimed

biopolymer marker (amino acid residues 2-12 of SEQ ID NO:1) is obtained from samples which have been isolated from a patient's body, thus the claimed biopolymer marker is "isolated" (see page 26, line 20 to page 27, line 2 and Figure 1 of the instant specification as originally filed).

Applicants' have now shown that the claimed invention is drawn to patentable subject matter and respectfully request that this rejection under 35 USC 101 be withdrawn.

Rejection under 35 USC 112, first paragraph (new matter)

Claims 41-43, as presented on May 16, 2005, remain rejected under 35 USC 112, first paragraph, as failing to comply with the written description requirement. The claims allegedly contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time that the application was filed, had possession of the claimed invention.

The Examiner indicates that the rejection in the previous Office Action mailed on November 14, 2003 is upheld. The instant specification recites that "the marker sequences of the present invention" may be used as antigens in immunoassays (page 31, lines 13-16). However, the Examiner asserts that there is no direct correlation of the "immunoassays" with a kit such as that recited

in claim 41. Immunoassays may be used in a plurality of devices and are not limited to use within a diagnostic kit. Specifically, a diagnostic kit comprising amino acid residues 2-12 of SEQ ID NO:1 is not recited in the specification and the specific page and line in the specification was not pointed to in the Arguments/Remarks filed on May 16, 2005. Therefore, the Examiner concludes that the diagnostic kit comprising the marker sequence consisting of amino acid residues 2-12 of SEQ ID NO:1 is deemed new matter.

Applicants respectfully disagree with the Examiner's assertions.

The "marker sequence" of the invention is explicitly disclosed as amino acid residues 2-12 of SEQ ID NO:1 in the instant specification as originally filed at page 27, lines 17-18; "...a disease specific marker identified by the sequence HRIHWESASLL." The sequence HRIHWESASLL is amino acid residues 2-12 of SEQ ID NO:1.

Additionally, the specification discloses that this "marker sequence" may be used as an antigen in immunoassays for the detection of those individuals suffering from the disease known to be evidenced by said marker sequence (see page 31, lines 13-16 of the instant specification as originally filed). In other words, amino acid residues 2-12 of SEQ ID NO:1 can be used in an immunoassay to identify individuals having myocardial infarction,

intracerebral hemorrhage or congestive heart failure. The specification lists several immunoassay formats, including the ELISA assay, that can be used (see page 31, lines 16-21 of the instant specification as originally filed). It is common for diagnostic tests, such as the ELISA, to be available commercially as kits; for example, from Immuno Diagnostics, Inc., see attached pages as accessed from their web site; reference 1). Thus, one of skill in art would likely assume that a diagnostic immunoassay, such as the one described at page 31 of the instant specification, is a kit.

Accordingly, Applicants contend that one of skill in the art would immediately recognize that diagnostic kits comprising the marker sequence consisting of amino acid residues 2-12 of SEQ ID NO:1 were contemplated by the inventors at the time that the application was filed.

Thus, Applicants respectfully submit that the limitation "diagnostic kit comprising the marker sequence consisting of amino acid residues 2-12 of SEQ ID NO:1" as recited in claims 41-43 does not constitute new matter.

Applicants have now addressed the Examiner's assertions regarding "new matter", and respectfully submit, as evidenced by the above arguments, that the instant specification conveys with reasonable clarity to those skilled in the art that, as of the

filing date, Applicants were in possession of the invention as now claimed (see MPEP 2163.02). Thus, Applicants respectfully request that this rejection under 35 USC 112, first paragraph be withdrawn.

Rejections under 35 USC 112, second paragraph

Claims 1 and 36, as presented on May 16, 2005, stand rejected under 35 USC 112, second paragraph, as being indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 recites "diagnostic for myocardial...heart failure (CHF)" which the Examiner asserts is non-essential descriptive subject matter. This phrase recites an inherent property of the peptide and does not further limit the structure itself. The Examiner asserts that this is deemed to be vague and indefinite.

Applicants respectfully disagree with the Examiner, as the phrase recites a use for the claimed marker, however, in the interest of compact, efficient prosecution, the phrase "diagnostic for myocardial infarction (MI), intracerebral hemorrhage (ICH) or congestive heart failure (CHF)" has been removed from claim 1.

Claim 36 step (b) recites "a manner effective to maximize elucidation of discernible peptide fragments" which the Examiner deems is vague and indefinite. The Examiner asserts that this is not a positive method step which clearly sets forth how this

particular method step is carried out. While the claims are read in light of the specification, limitations from the specification cannot be read into the claims. The Examiner asserts that the entire phrase appears unnecessary, as it is unclear what such a "manner", as stated specifically in claim 36 would be.

Applicants respectfully disagree with the Examiner's conclusions as the "manner" is a distinguishing step of the claimed method, and as such, is in no way "unnecessary".

Prior art mass spectrometric techniques often failed to identify the entire range of proteins present in a sample; i.e. the proteins present in the greatest amounts were most easily identified (see page 11, lines 1-14 of the instant specification as originally filed). The claimed method overcomes this limitation of the prior art by the use of preparatory steps, such as chromatography, prior to analysis of a sample using mass spectrometric techniques. These preparatory steps help to maximize the diversity of biopolymers discernible in a sample; i.e. the preparatory steps of the instant invention allow the identification of more of the biopolymers(including the less abundant proteins) present in a sample than previous methods allow. See, for example, the abstract and page 20, lines 7-12 of the instant specification as originally filed. Furthermore, pages 20-25 specifically disclose many of the preparatory chromatographic protocols used to carry out

the claimed method. Thus, it is clear from the disclosure that the phrase "manner effective to maximize" refers to the preparatory steps (to mass spectrometry) of the claimed method.

The Examiner further asserts that claim 36(c) recites wherein "recognition of a mass spectrum profile...displaying the characteristic profile of the mass spectrum profile" which is deemed to be vague and indefinite. The claim language seems unnecessarily wordy and confusing as to the distinction between various mass spectrum profiles recited in said claim. Additionally, the term "recognition" implies a process in the human brain, and not a computerized step. The profile does not "display" anything, "characteristic profile" is a relative term which appears to be changeable, and the end of the claim sets forth "is diagnostic.." which again (as above) is unessential descriptive subject matter.

Applicants respectfully submit that the Examiner's comments reveal an incomplete understanding of the claimed method.

Step (b) of claim 36, as amended herein, involves comparing the mass spectral profile of the biopolymer marker consisting of amino acid residues 2-12 of SEQ ID NO:1 (profile shown in Figure 2) to mass spectral profiles obtained from an unknown sample. It is important to point out that mass spectral profiles are reproducible; many have been published and may be used as references for identification of unknowns (see attached article

"Introduction to Mass Spectrometry" accessed from the web site of the University of Arizona Department of Chemistry; reference 2). Thus, the instant invention provides a mass spectral profile of a biopolymer marker for myocardial infarction, intracerebral hemorrhage or congetive heart failure, Figure 2, which is intended to be used as a reference to determine the presence of the biopolymer marker in unknown samples.

Step (c) of claim 36, as amended herein, indicates that the mass spectral profile of the claimed biopolymer marker displays an ion peak at about 1348 daltons and that the presence of the biopolymer marker is confirmed by the identification of this ion peak in a mass spectral profile obtained from a sample. The peak at 1348 daltons is the characteristic mass spectral profile of the claimed biopolymer marker (amino acid residues 2-12 of SEQ ID NO:1). Identification of this mass spectral profile links the sample to myocardial infarction, intracerebral hemorrhage and congestive heart failure, for example, if the mass spectral profile shown in Figure 2 is obtained from an unknown sample, the presence of the claimed biopolymer marker is confirmed and thus, represents a diagnosis of myocardial infarction, intracerebral hemorrhage or congestive heart failure.

The Examiner suggests steps which clearly a)obtain a sample; b) perform mass spectrometry; c) identify the presence or absence

of the peptide consisting of amino acid residues 2-12 of SEQ ID NO:1 and diagnose myocardial infarction...based on the presence of said peptide in the sample.

However, the Examiner's suggestion is incomplete as it does not include all of the elements of the claimed method, for example, the preparatory chromatographic steps.

Accordingly, Applicants have now clarified the metes and bounds of the claims and respectfully request that the above-discussed rejection under 35 USC 112, second paragraph be withdrawn.

CONCLUSION

In light of the foregoing remarks and amendments to the claims, it is respectfully submitted that the Examiner will now find the claims of the application allowable. Favorable reconsideration of the application is courteously requested.

Respectfully submitted,

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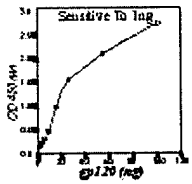
2132.030
Examiner copy
reference # 1

Immuno Diagnostics, Inc.

Products for HIV & Human T-Cell Research/Diagnostics
Cytokines/Lymphokines and Growth Factors
HIV/SIV Diagnostic Testing Kits

MENU

gp120 Capture ELISA



PRODUCT# *101* $495.00

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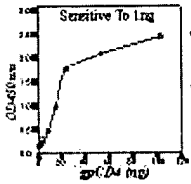
CD4 Capture ELISA



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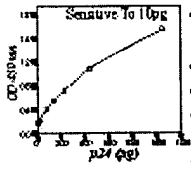
p24 Capture ELISA



PRODUCT# *103* $495.00

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NOTE:
Specifications are in Acrobat PDF Format - click icon to right to get the FREE reader... link will open in new window

- **HIGHLY SENSITIVE:** ImmunoAssay Kits are thoroughly tested to meet the highest sensitivity standards, guaranteed.
- **COMPLETE KIT:** Each kit comes complete with all the reagents needed to perform the Assay, including positive/negative controls.
- **EASY TO USE:** Each kit includes a comprehensive, step by step instruction manual. All procedures are explained in detail.
- **LOW COST:** ImmunoAssay Kits are priced to fit any research budget. Bulk/blanket order discounts available upon request.

a132.030
Examiner copy
reference #2

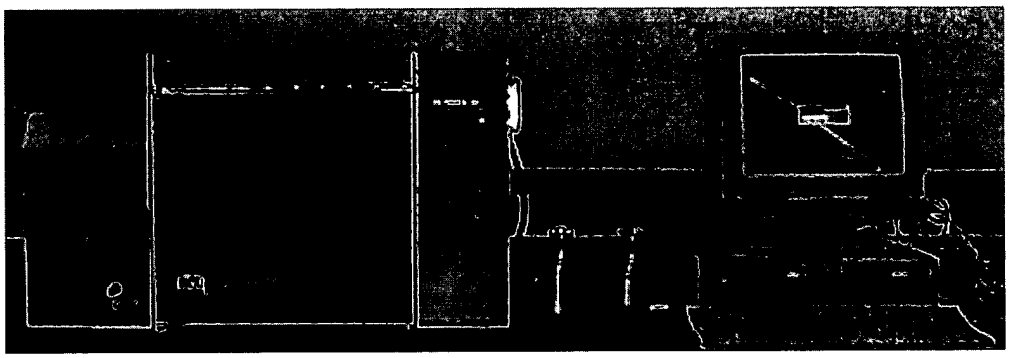
THE UNIVERSITY OF ARIZONA
Department of Chemistry

Introduction to Mass Spectrometry

Contents of this introduction:

- ***GC/MS:*** *a description of the instrument*
- ***Interpreting Spectra:*** *what information does a mass spectrum provide?*
- ***Other methods:*** *other mass spectrometry techniques are described*
- ***Websites:*** *where you can go for more mass spectrometry information*

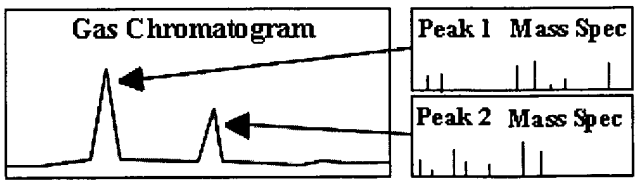
GC/MS



A mass spectrometer creates charged particles (ions) from molecules. It then analyzes those ions to provide information about the molecular weight of the compound and its chemical structure. There are many types of mass spectrometers and sample introduction techniques which allow a wide range of analyses. This discussion will focus on mass spectrometry as it's used in the powerful and widely used method of coupling Gas Chromatography (GC) with Mass Spectrometry (MS).

Pictured above is a GC/MS instrument used in the organic teaching labs.

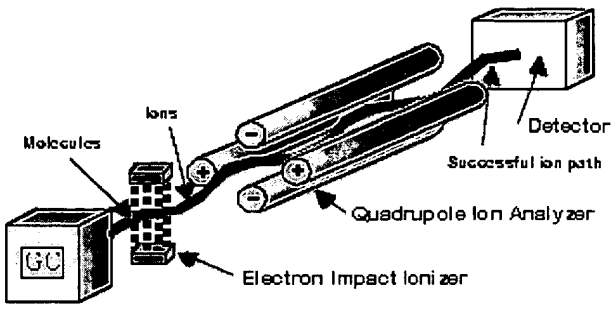
Gas Chromatograph (GC)



A mixture of compounds to be analysed is initially injected into the GC where the mixture is vaporized in a heated chamber. The gas mixture travels through a GC column, where the compounds become separated as they interact with the column. The chromatogram on the right shows peaks which result from this separation. Those separated compounds then immediately enter the mass spectrometer.

Mass Spectrometer (MS)

Below is a general schematic of a mass spectrometer. The blue line illustrates ions of a particular mass/charge ratio which reach the detector at a certain voltage combination.



1) Ionizer 2) Ion Analyzer 3) Detector

Ionizer

In the GC-MS discussed in this introduction, the charged particles (ions) required for mass analysis are formed by Electron Impact (EI) Ionization. The gas molecules exiting the GC are bombarded by a high-energy electron beam (70 eV). An electron which strikes a molecule may impart enough energy to remove another electron from that molecule. Methanol, for example, would undergo the following reaction in the ionizing region:

CH_3OH + 1 electron ⟶ $CH_3OH^{+\cdot}$ + 2 electrons

(note: the symbols $^{+\cdot}$ indicate that a radical cation was formed)

EI Ionization usually produces singly charged ions containing one unpaired electron. A charged molecule which remains intact is called the molecular ion. Energy imparted by the electron impact and, more importantly, instability in a molecular ion can cause that ion to break into smaller pieces (fragments). The methanol ion may fragment in various ways, with one fragment carrying the charge and one fragment remaining uncharged. For example:

$CH_3OH^{+\cdot}$(molecular ion) ⟶ CH_2OH^{+}(fragment ion) + $H^{\cdot}$

(or) $CH_3OH^{+\cdot}$(molecular ion) ⟶ CH_3^{+}(fragment ion) + $^{\cdot}OH$

Ion Analyzer

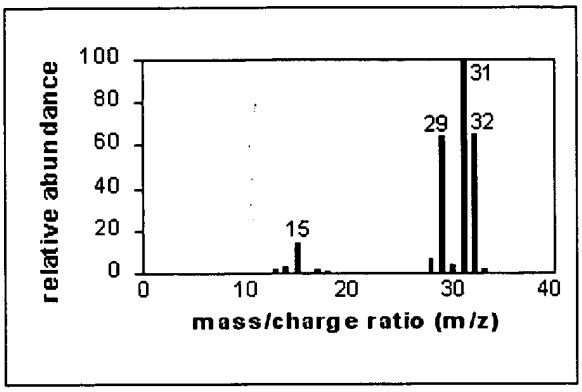
Molecular ions and fragment ions are accelerated by manipulation of the charged particles through the mass spectrometer. Uncharged molecules and fragments are pumped away. The quadrupole mass analyzer in this example uses positive (+) and negative (-) voltages to control the path of the ions. Ions travel down the path based on their mass to charge ratio (m/z). EI ionization produces singly charged particles, so the charge (z) is one. Therefore an ion's path will depend on its mass. If the (+) and (-) rods shown in the mass spectrometer schematic were ?fixed' at a particular rf/dc voltage ratio, then one particular m/z would travel the successful path shown by the solid line to the detector. However, voltages are not fixed, but are scanned so that ever increasing masses can find a successful path through the rods to the detector.

Detector

There are many types of detectors, but most work by producing an electronic signal when struck by an ion. Timing mechanisms which integrate those signals with the scanning voltages

allow the instrument to report which m/z strikes the detector. The mass analyzer sorts the ions according to m/z and the detector records the abundance of each m/z. Regular calibration of the m/z scale is necessary to maintain accuracy in the instrument. Calibration is performed by introducing a well known compound into the instrument and "tweaking" the circuits so that the compound's molecular ion and fragment ions are reported accurately.

Interpreting spectra



ions	m/z
$CH_3OH^{+\cdot}$	32
$H_2C{=}OH^+$	31
$HC{\equiv}O^+$	29
H_3C^+	15

A simple spectrum, that of methanol, is shown here. $CH3OH^{+\cdot}$ (the molecular ion) and fragment ions appear in this spectrum. Major peaks are shown in the table next to the spectrum. The x-axis of this bar graph is the increasing m/z ratio. The y-axis is the relative abundance of each ion, which is related to the number of times an ion of that m/z ratio strikes the detector. Assignment of relative abundance begins by assigning the most abundant ion a relative abundance of 100% ($CH2OH^+$ in this spectrum). All other ions are shown as a percentage of that most abundant ion. For example, there is approximately 64% of the ion CHO^+ compared with the ion $CH2OH^+$ in this spectrum. The y-axis may also be shown as abundance (not relative). Relative abundance is a way to directly compare spectra produced at different times or using different instruments.

EI ionization introduces a great deal of energy into molecules. It is known as a "hard" ionization method. This is very good for producing fragments which generate information about the structure of the compound, but quite often the molecular ion does not appear or is a smaller peak in the spectrum.

Of course, real analyses are performed on compounds far more complicated than methanol. Spectra interpretation can become complicated as initial fragments undergo further fragmentation, and as rearrangements occur. However, a wealth of information is contained in a mass spectrum and much can be determined using basic organic chemistry "common sense".

Following is some general information which will aid EI mass spectra interpretation:

Molecular ion ($M^{\cdot+}$): If the molecular ion appears, it will be the highest mass in an EI spectrum (except for isotope peaks discussed below). This peak will represent the molecular weight of the compound. Its appearance depends on the stability of the compound. Double bonds, cyclic structures and aromatic rings stabilize the molecular ion and increase the probability of its appearance.

Reference Spectra: Mass spectral patterns are reproducible. The mass spectra of many compounds have been published and may be used to identify unknowns. Instrument computers generally contain spectral libraries which can be searched for matches.

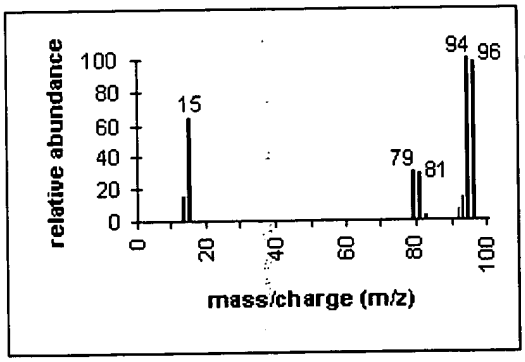
Fragmentation:General rules of fragmentation exist and are helpful to predict or interpret the fragmentation pattern produced by a compound. Functional groups and overall structure determine how some portions of molecules will resist fragmenting, while other portions will fragment easily. A detailed discussion of those rules is beyond the scope of this introduction, and further information may be found in your organic textbook or in mass spectrometry reference books. A few brief examples by functional group are described (see examples).

Isotopes:Isotopes occur in compounds analyzed by mass spectrometry in the same abundances that they occur in nature. A few of the isotopes commonly encountered in the analyses of organic compounds are below along with an example of how they can aid in peak identification.

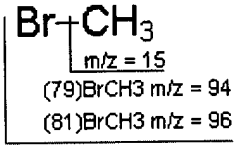
Relative Isotope Abundance of Common Elements:

Element	Isotope	Relative Abundance	Isotope	Relative Abundance	Isotope	Relative Abundance
Carbon	^{12}C	100	^{13}C	1.11		
Hydrogen	^{1}H	100	^{2}H	.016		
Nitrogen	^{14}N	100	^{15}N	.38		
Oxygen	^{16}O	100	^{17}O	.04	^{18}O	.20
Sulfur	^{32}S	100	^{33}S	.78	^{34}S	4.40
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98.0

Methyl Bromide: An example of how isotopes can aid in peak identification.



The ratio of peaks containing ^{79}Br and its isotope ^{81}Br (100/98) confirms the presence of bromine in the compound.



Other Methods

An array of ionization methods and mass analyzers are available to meet the needs of many types of chemical analysis. A few are listed here with a highlight of their usefulness.

Sample introduction/ionization method:

Ionization method	Typical Analytes	Sample Introduction	Mass Range	Method Highlights
Electron Impact (EI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Hard method versatile provides structure info
Chemical Ionization (CI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Soft method molecular ion peak $[M+H]^+$
Electrospray (ESI)	Peptides Proteins nonvolatile	Liquid Chromatography or syringe	to 200,000 Daltons	Soft method ions often multiply charged
Fast Atom Bombardment (FAB)	Carbohydrates Organometallics Peptides nonvolatile	Sample mixed in viscous matrix	to 6,000 Daltons	Soft method but harder than ESI or MALDI
Matrix Assisted Laser Desorption (MALDI)	Peptides Proteins Nucleotides	Sample mixed in solid matrix	to 500,000 Daltons	Soft method very high mass

Mass Analyzers:

Analyzer	System Highlights
Quadrupole	Unit mass resolution, fast scan, low cost
Sector (Magnetic and/or Electrostatic)	High resolution, exact mass
Time-of-Flight (TOF)	Theoretically, no limitation for m/z maximum, high throughput
Ion Cyclotron Resonance (ICR)	Very high resolution, exact mass, perform ion chemistry

Linked Systems:

GC/MS:	Gas chromatography coupled to mass spectrometry
LC/MS:	Liquid chromatography coupled to electrospray ionization mass spectrometry

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Useful websites

A library of spectra can be found in the NIST WebBook, a data collection of the National Institute of Standards and Technology.

Useful tools such as an exact mass calculator and a spectrum generator can be found in the MS Tools section of Scientific Instrument Services webpage.

The JEOL Mass Spectrometry website contains tutorials, reference data and links to other sites.

More general information and tutorials can be found in Scimedia, an educational resource.

At the University of Arizona, the Wysocki Research Group studies surface-induced dissociation (SID) tandem mass spectrometry.

Many more interesting and useful links can be found by following the site links in the above references.

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If you have comments or suggestions, email me at *breci@u.arizona.edu*